

AD-A152 289

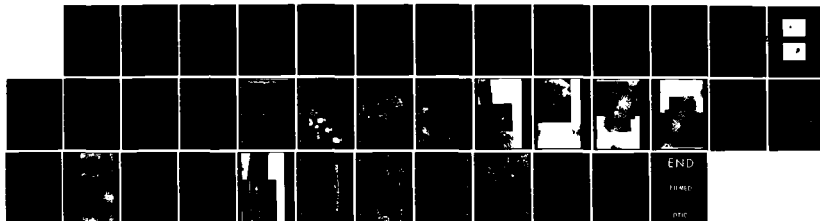
SEQUENTIAL MORPHOLOGIC ALTERATIONS IN THE FOVEOLA AND
CORNEA OF NONHUMAN. (U) PACIFIC MEDICAL CENTER SAN
FRANCISCO CA W H SPENCER MAR 83 DAMD17-79-C-9132

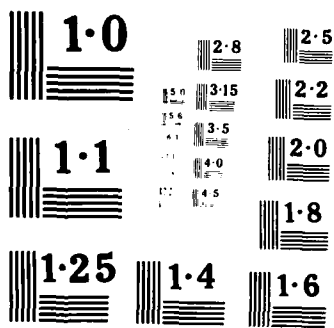
1/1

UNCLASSIFIED

F/G 20/5

NL





AD-A152 289

DTIC ACCESSION NUMBER

II

LEVEL

PHOTOGRAPH THIS SHEET

1

INVENTORY

DAMD 17-79-C-9132 - Annual Rept.
DOCUMENT IDENTIFICATION March 1983

DISTRIBUTION STATEMENT A

Approved for public release;
Distribution Unlimited

DISTRIBUTION STATEMENT

ACCESSION FOR

NTIS GRA&I ☒

DTIC TAB ☐

UNANNOUNCED ☐

JUSTIFICATION

BY

DISTRIBUTION

AVAILABILITY CODES

DIST

AVAIL AND/OR SPECIAL

A-1

DISTRIBUTION STAMP

Copy available to DTIC does not
represent full DTIC reproduction

DTIC
ELECT
APR 5 1985
S D

DATE ACCESSIONED

1. contains color
2. DTIC reproduction
3. in black and

DATE RETURNED

85 4 04 612
DATE RECEIVED IN DTIC

REGISTERED OR CERTIFIED NO.

PHOTOGRAPH THIS SHEET AND RETURN TO DTIC-DDAC

DISCLAIMER NOTICE

THIS DOCUMENT IS BEST QUALITY PRACTICABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

Sequential Morphologic Alterations in the Foveola and Cornea
of Nonhuman Subjects After Exposure to Coherent Light

Annual Progress Report

William H. Spencer, M. D.

March 1983

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-79-C-9132

Pacific Medical Center
2340 Clay Street
San Francisco, CA 94115

DOD Distribution Statement

Approved for public release,
distribution unlimited

The findings in this report are not to be construed as an official Department
of the Army position unless so designated by other authorized documents.

TABLE OF CONTENTS

	Page Number
Report Documentation Page	1, 2
Summary	3
Foreword	4
Report	5
Background.	5
Scope of Work	5
Rhesus Monkey Studies	6
Procedure	6
Location of Marker & Experimental Lesions	6
Figure 1	7
Figure 2	8
Figure 3	9
Morphology of Marker & Experimental Lesions	10
Marker Lesions	10
Retinal Pigment Epithelium	10
Outer Segments of Rods & Cones	10
Inner Segments of Rods & Cones	11
Outer Plexiform Layer	11
Experimental Lesions	11
Experimental Lesions - Left eye	11
Outer Segments of Rods & Cones	11
Inner Segments of Rods & Cones	11
Figure 4	12
Figure 4B.	13
Figure 5.	14
Figure 6	15
Figure 6A.	16
Figure 7, 7A	17, 18
Figure 8	19
Figure 8A	20
Figure 9	21

TABLE OF CONTENTS

	Page Number
Figure 9A	22
Outer Plexiform Layer.	23
Central Foveolar Lesion	23
Experimental Lesion, Right Eye	23
Discussion of Results	23, 24
Figure 10	24
Figure 10A	25
Figure 10B	26
Figure 11.	27, 28
Recommendations	29

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Sequential Morphologic Alterations in the Foveola and Cornea of Nonhuman Subjects After Exposure to Coherent Light		5. TYPE OF REPORT & PERIOD COVERED 1 October 1981 to 15 March 1983
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) William H. Spencer, M. D.		8. CONTRACT OR GRANT NUMBER(s) DAMD17-79-C-9132
9. PERFORMING ORGANIZATION NAME AND ADDRESS Pacific Presbyterian Medical Center 2340 Clay Street San Francisco, California 94115		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62772A.3E162772A878.BA.204
11. CONTROLLING OFFICE NAME AND ADDRESS U. S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND Fort Detrick, Frederick, Maryland 21701-5012		12. REPORT DATE March 1983
		13. NUMBER OF PAGES 29
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Illum arsenide laser; Focal retinal lesions; Intracardiac perfusion Marker and Experimental lesion		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Serial light microscopic and ultrastructural sections were prepared through well-fixed marker and experimental macular retinal lesions in both eyes of a rhesus monkey, sacrificed seven days after exposure to a neodymium-YAG dye laser tuned to 900 nm (gallium arsenide). Each laser exposure was 50 microns in diameter and of 20 nanosecond pulse duration. The marker lesions were produced by 120 pulses (average 30.6 microjoule/pulse) and the experimental lesions by a single 30.6 microjoule pulse (average) in the left eye and a single 9.5 microjoule pulse (average) in the right eye. The morphologic changes in the marker		

and experimental lesions were qualitatively similar but differed in the extent of tissue response. The left eye experimental single pulse laser exposures showed histologic evidence of significant tissue disruption, primarily involving the retinal pigment epithelium, outer segments of the rods and cones and outer nuclear layer. The experimental lesions in the right eye contained only equivocal histologic evidence of tissue damage, suggesting either that a 20 nanosecond 50 micron diameter laser exposure of 9.5 microujoules is not of sufficient magnitude to produce a lesion, or that the lesions were present but missed.

2) At the request of LAIR personnel, the contractor prepared light and electron microscopic slides of turtle eye cup preparations and one laser irradiated calcium-cadmium laser turtle retinal eye cup preparation. This work was performed as part of an investigation by Dr. Michael Rayburn at the Lawrence Berkeley Laboratory at the University of California.

SUMMARY

This investigation of the parameters of the damaging effects of coherent light upon the primate retina was initiated because of concern that the eyes of military or civilian personnel might inadvertently be exposed to this form of energy causing transient or permanent visual loss. The effects of coherent light upon ocular tissues are known to vary with the physical characteristics of the energy exposure (wavelength, duration of pulse, spot size, amplitude). This study examines the morphologic effects upon the retina of coherent light at a wavelength of 900 nm (gallium arsenide). This wavelength is similar to that used in range finders and target designators. At the request of LAIR personnel this contractor also prepared light and electron microscopic sections through turtle retina eye cup preparations as part of an investigation by Dr. Michael Raybourn at the Lawrence Berkeley Laboratory of the University of California. The latter studies are separately reported by Dr. Raybourn.

Our study of the effects of coherent light at 900 nm upon the primate retina show that a single 20 nanosecond, 50 micron diameter, 30.6 microjoule/pulse of coherent light produces significant and consistent tissue damage. However, a single 9.5 microjoule/pulse of similar dimension and duration will not cause a histologically demonstrable lesion. The total intraocular energy necessary to produce a lesion in 50% of exposed retinas is presumed to lie between 9.5 and 30.6 microjoules per pulse. This investigation delineates the tissue alterations present at 7 days after laser exposure. A better assessment of the dimensions of the immediate visual perturbation and the changes that occur during the first week after exposure could be obtained by repeating this investigation at appropriate short and intermediate term intervals after exposure. This will provide data regarding the reparative tissue responses, the potential for recovery of visual function and its timing.

FOREWORD

In conducting the research described in this report the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-83), Revised 1978).

REPORT

This is a report of an investigation performed at the Eye Pathology Laboratory of the Pacific Presbyterian Medical Center (PPMC) as a joint effort with personnel of the Division of Biorheology at the Letterman Army Institute of Research (LAIR). The study is designed to evaluate and characterize the morphologic effects of exposure of rhesus monkey retina to discretely applied short duration single and multiple pulse coherent light in the 900 nm (gallium arsenide) wavelength range. The report also includes a summary of work performed at the request of LAIR in coordination with Dr. Michael Raybourn of the Lawrence Berkeley Laboratory at the University of California who is investigating the electrophysiologic and morphologic effects of gallium arsenide laser energy upon the turtle retina.

BACKGROUND

In order to provide a base upon which safety standards may be established for humans, the Division of Biorheology at LAIR has conducted a series of investigations in nonhuman subjects of the functional and morphologic effects of coherent and incoherent light upon ocular tissue. Mechanical, thermal and photochemical retinal damage is known to be produced by exposure to optical sources that produce a discrete image upon the retina at wavelengths between 400 and 1400 nm. The extent of tissue damage produced depends upon the energy level, its wavelength and the duration of its exposure. It is possible that the eyes of military personnel may become exposed to coherent light at wavelengths and intensities similar to those used in this investigation. This study will assist in quantifying the effects of inadvertent momentary ocular exposure by individuals using laser systems such as range finders and target designators. It is designed to evaluate and characterize the morphologic effect within the rhesus monkey retina, at the light and electron microscopic level of a short duration, small diameter, low energy single pulse exposure to coherent light in the gallium arsenide wavelength range. Because the effects of these small lesions are difficult to discern with the ophthalmoscope and at the time of gross examination of the specimen, a pattern of more readily visualized larger marker lesions was placed in the adjacent retina to facilitate orientation.

SCOPE OF WORK

This section describes work performed in accordance with the protocol of this contract. In addition this contractor prepared (at the request of LAIR personnel and of Dr. Michael Raybourn of the Lawrence Berkeley Laboratory at the University of California (Project Order: 2804) electron microscopic sections through three un-irradiated turtle retina eye cup preparations and one irradiated (helium-cadmium laser at 441 nm) turtle preparation. Dr. Raybourn has been investigating the electrophysiologic and morphologic effects produced by gallium arsenide laser irradiation (900 nm) in the turtle retina. This investigation requires electrophysiologic measurements from these retinas up to 2 hours after enucleation.

We prepared sections through turtle retina fixed immediately after enucleation, 40 minutes after enucleation, and 2 hours after enucleation. These showed surprisingly little morphologic evidence of artifact resulting from delayed fixation. The fourth turtle retina had been exposed to focal laser irradiation in the helium-cadmium wavelength range (441 nm). These exposures were placed for purposes of orientation ("calibrating" lesions) with respect to the area of retina to be irradiated with the gallium arsenide laser. These lesions were sectioned and found to show oil droplet loss and lamellar disarray in the photoreceptors. Photographs of all tissues studied were delivered to Dr. Raybourn for inclusion in this report.

RHESUS MONKEY STUDIES - PRELIMINARY PROCEDURES

In preparation for the planned investigations of a rhesus monkey retina, tissue was studied from a Cebus monkey sacrificed at an outside facility after intraventricular perfusion with triple fixative. The tissue was embedded in Epon and the prepared sections compared with sections from similar areas of rhesus monkey retina fixed by immersion and processed in Epon-Araldite. The fixation and embedding of the Cebus retina was felt to be a marked improvement over that obtained by the immersion technique utilized for the rhesus retinas.

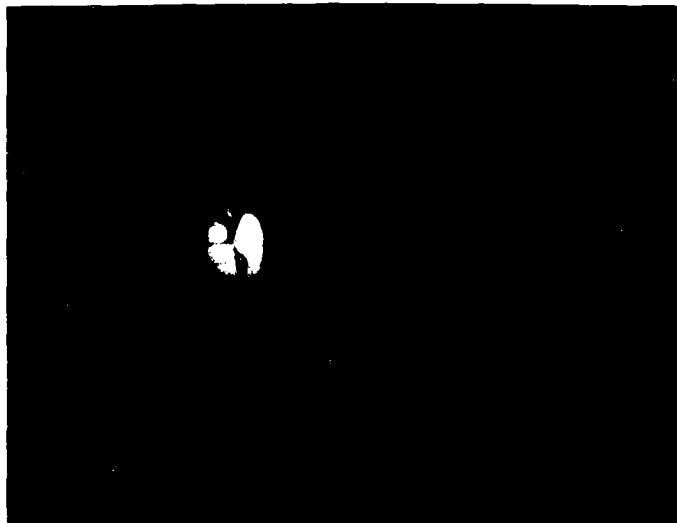
PROCEDURE

Marker and experimental lesions were placed in both retinas of a rhesus monkey (R336) by personnel at LAIR and the location of the marker lesions were documented by polaroid fundus photographs. The animal was sacrificed seven days following exposure and the tissue immediately fixed via a combination of intracardiac perfusion and intraocular injection of triple fix solution. Following gross dissection, embedding and sectioning, the tissue was found to be very well fixed with excellent definition of ultrastructural detail. A large number of serial sections through the macular region of both eye were then prepared. These delineate the extent of the morphologic alterations within the marker and experimental lesions and constitute the basis for this report.

LOCATION OF MARKER & EXPERIMENTAL LESIONS - PARAMETERS OF INCIDENT COHERENT LIGHT

Marker and experimental lesions were placed by LAIR personnel in the macular region of each retina of R336 in the pattern shown in the attached polaroid photographs (Fig. 1) and diagram (Fig. 2). The lesions were produced by a neodymium-YAG laser tuned to 900 nm (gallium arsenide). Each laser exposure was 50 microns in diameter and of 20 nanosecond pulse duration. The average total incident energy (TIE) for the marker lesions in each eye and for the experimental lesions in the left eye was 30.6 microjoules/pulse. The TIE for the experimental lesions in the right eye averaged 9.5 microjoules/pulse (Fig. 3). The marker lesions were produced by 120 pulses and the experimental lesions by a single pulse.

R336 (DR SPENCER) 20 SEPT 82 O.S.



R336 (DR SPENCER) 20 SEPT 82 O.D.



FIGURE 1 MARKER AND EXPERIMENTAL LESIONS

MARKER AND EXPERIMENTAL LESIONS WERE PLACED BY LAIR PERSONNEL IN THE MACULAR REGION OF EACH RETINA OF R336 IN THE PATTERN SHOWN IN THE POLAROID PHOTOGRAPHS.

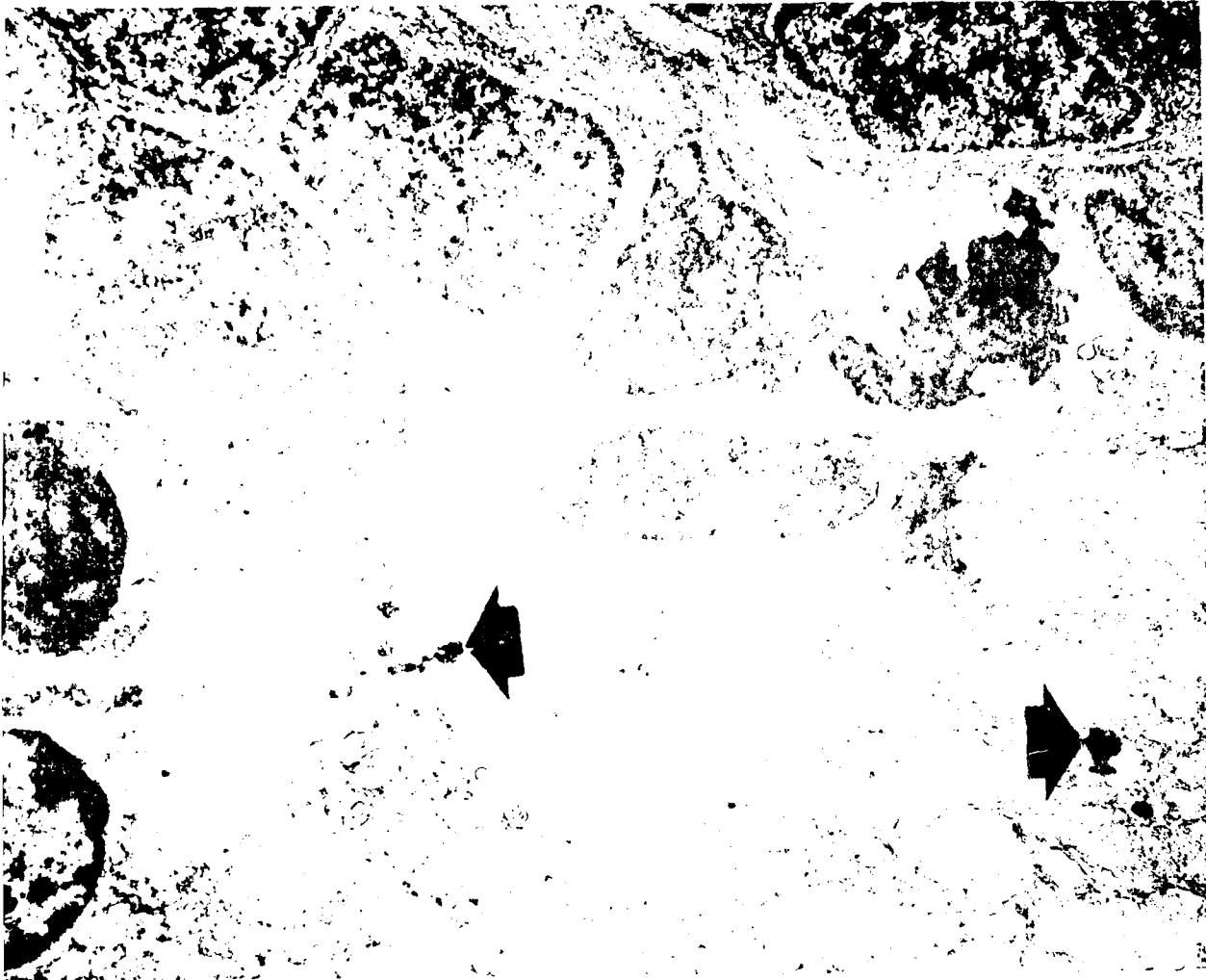


FIGURE 8A

7,500X

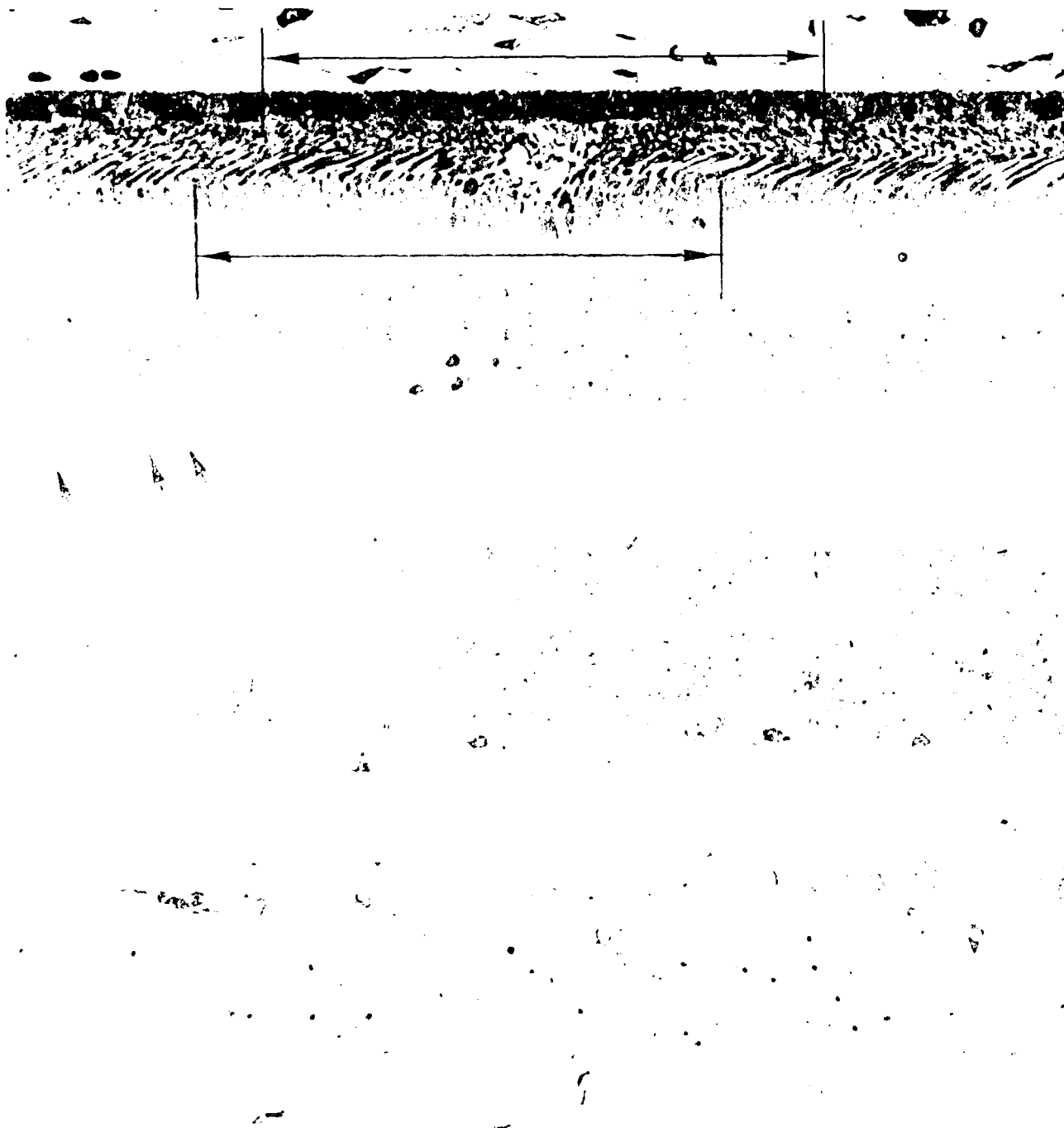


FIGURE 8

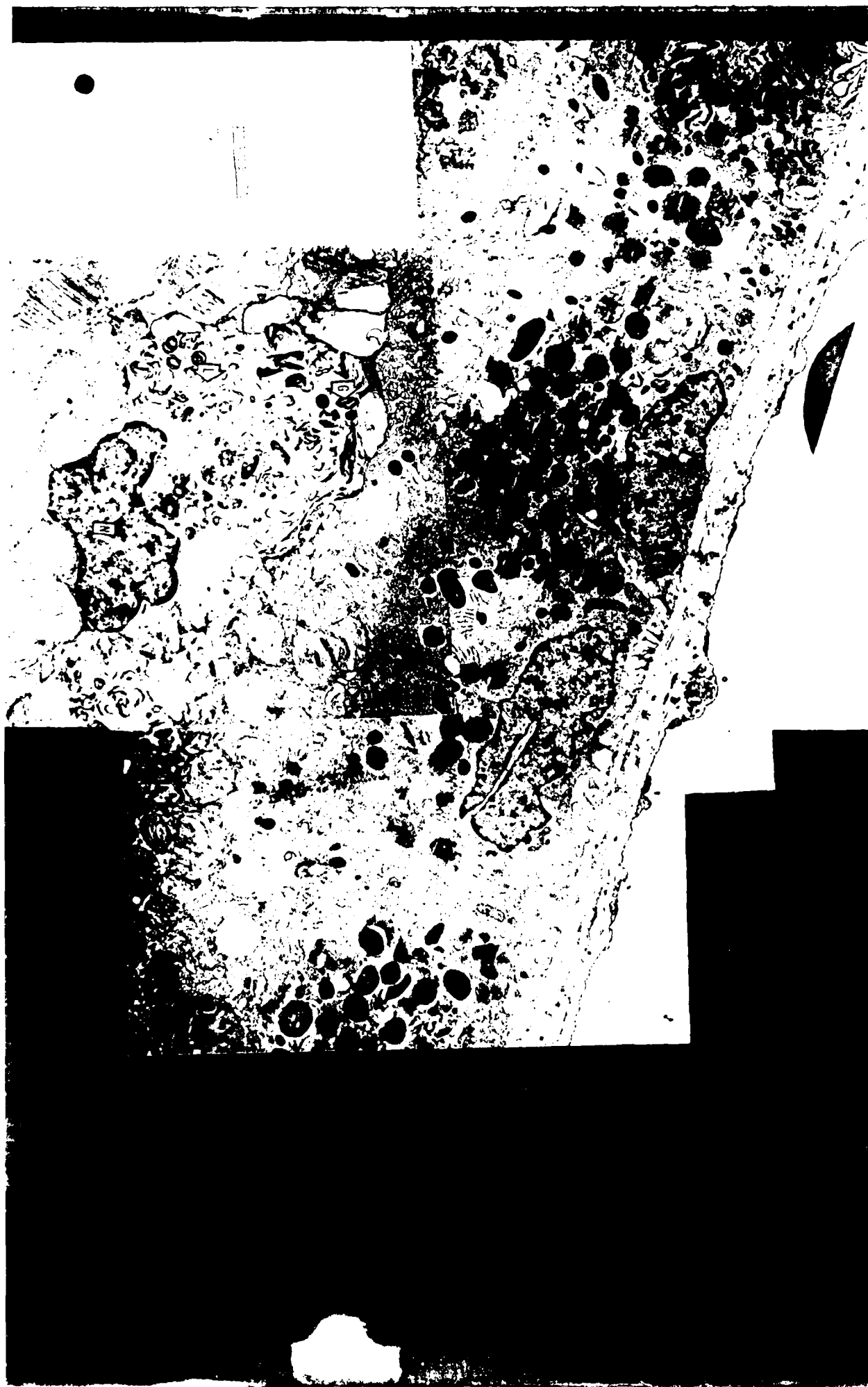
500X

FIGURE 8 MARKER LESION LEFT EYE

NOTE DARK STAINING IN AXONS OF OUTER PLEXIFORM LAYER (ARROWHEADS).
FIGURE 8A PROVIDES HIGHER MAGNIFICATION VIEW DEMONSTRATING INTRA-
AXONAL DEBRIS.

BRACKETED AREAS DEPICT ZONES OF RPE OUTER SEGMENT AND OUTER NUCLEAR
LAYER INVOLVEMENT.





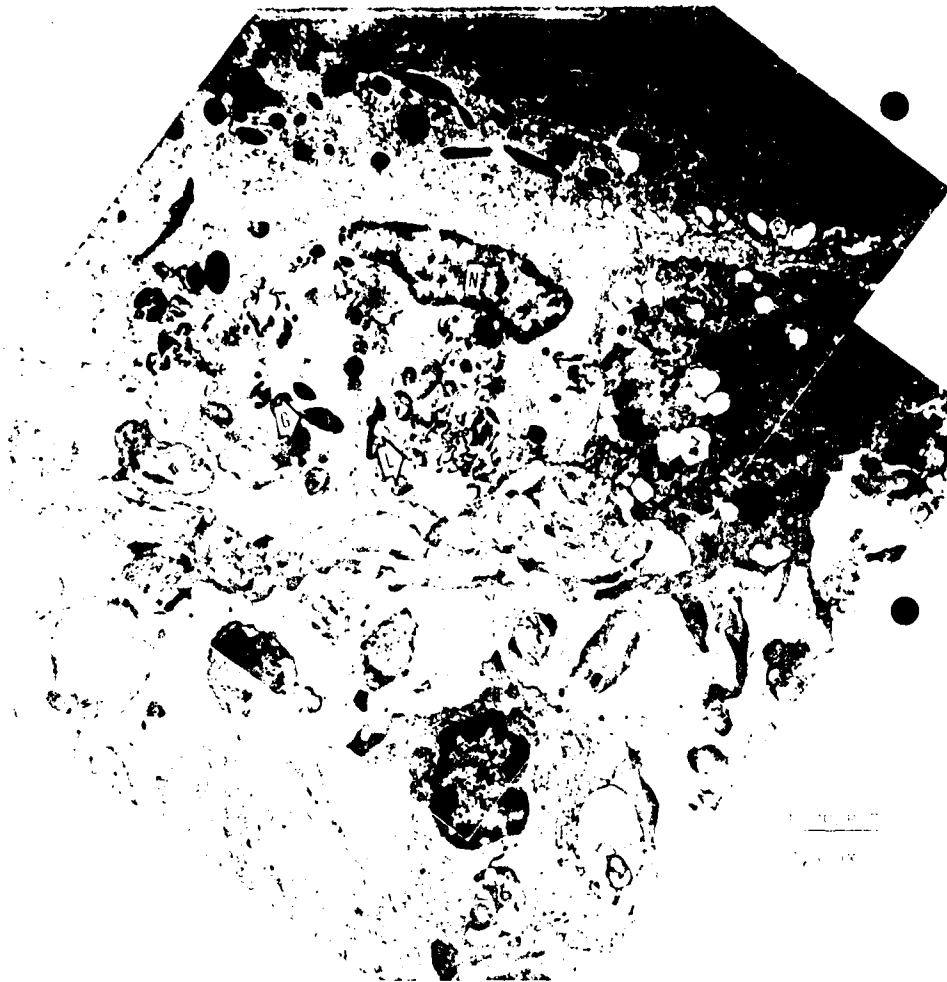


FIGURE 1
LOW POWER VIEW

FIGURE 1. LOW POWER VIEW OF TISSUE

ON THE LEFT, THE TISSUE IS SEPARATED FROM THE PLEURAL EPITHELIUM
 WITHIN THE PLEURAL SPACE. ON THE RIGHT, A LOW POWER VIEW
 OF THE TISSUE IS SHOWN.

ON THE LEFT, THE TISSUE IS SEPARATED FROM THE PLEURAL EPITHELIUM
 WITHIN THE PLEURAL SPACE. ON THE RIGHT, A LOW POWER VIEW
 OF THE TISSUE IS SHOWN.

ON THE LEFT, THE TISSUE IS SEPARATED FROM THE PLEURAL EPITHELIUM
 WITHIN THE PLEURAL SPACE. ON THE RIGHT, A LOW POWER VIEW
 OF THE TISSUE IS SHOWN.



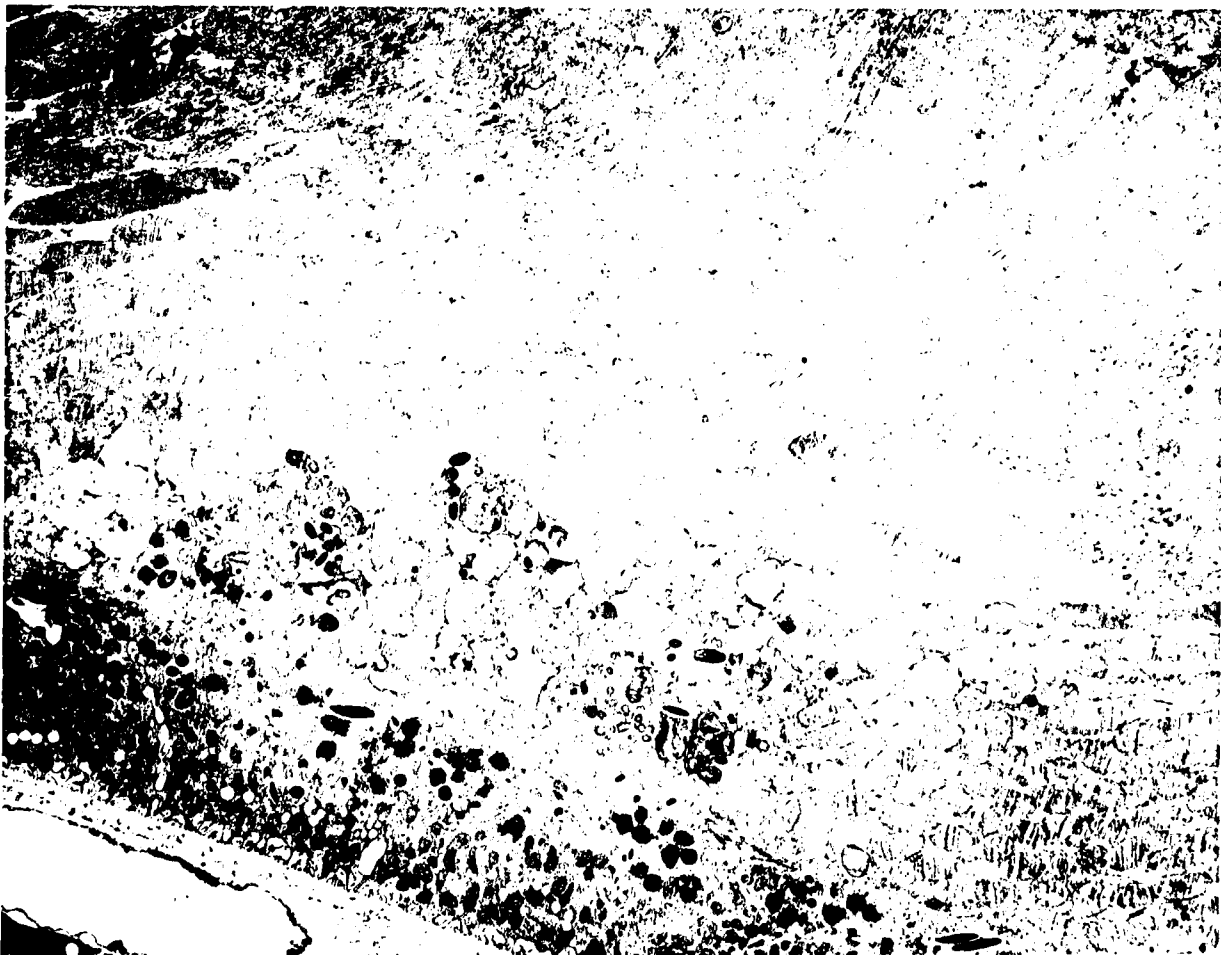
DISRUPTION AND DISTORTION OF ROD AND CONE OUTER SEGMENTS.
SHOWN IN HIGHER MAGNIFICATION IN FIGURE 6A.

MARKER LESION LEFT EYE

FIGURE 6

2,500X

FIGURE 6



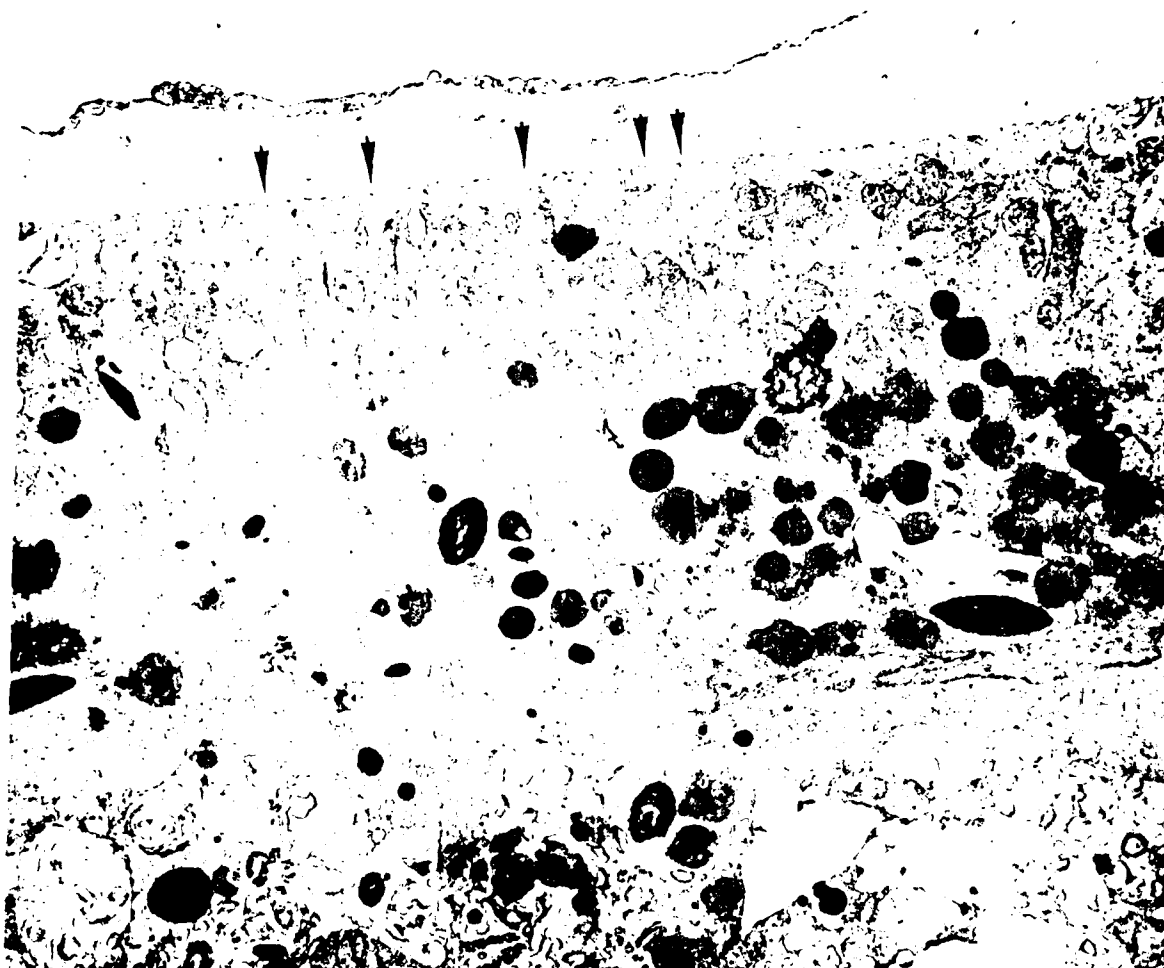


FIGURE 5

7,500X

FIGURE 5 MARKER LESION LEFT EYE

SMALL ARROWHEADS POINT TO APPARENTLY NORMAL RELATIONSHIP BETWEEN BASAL INFOLDINGS OF INTRALESIONAL RPE CELLS AND THE PIGMENT EPITHELIAL BASEMENT MEMBRANE. LARGE ARROWHEADS POINT TO APPARENT WIDENING OF INTERCELLULAR INTERFACE SEEN ONLY BETWEEN OCCASIONAL CELLS.

IN THIS PHOTOGRAPH THE WIDTH OF THE RPE ABNORMALITY MEASURES
 APPROXIMATELY 150 μ . PHOTO 4A AT HIGHER MAGNIFICATION SHOWS
 FLATTER RPE CELLS WITH LOSS OF APICAL PIGMENT GRANULES.
 ARROWHEADS POINT TO DISPLACED RPE CELLS WITHIN OUTER SEGMENTS.
 AREA DEPICTED IN OUTER NUCLEAR LAYER (BETWEEN ARROWHEADS) SHOWS
 OUTWARD BOWING AND NUCLEAR PYKNOSIS. THE LATTER IS SHOWN AT
 HIGHER MAGNIFICATION IN PHOTO 4B (LARGER ARROWHEADS).

13.

MARKER LESION LEFT EYE

FIGURE 4

7,500X

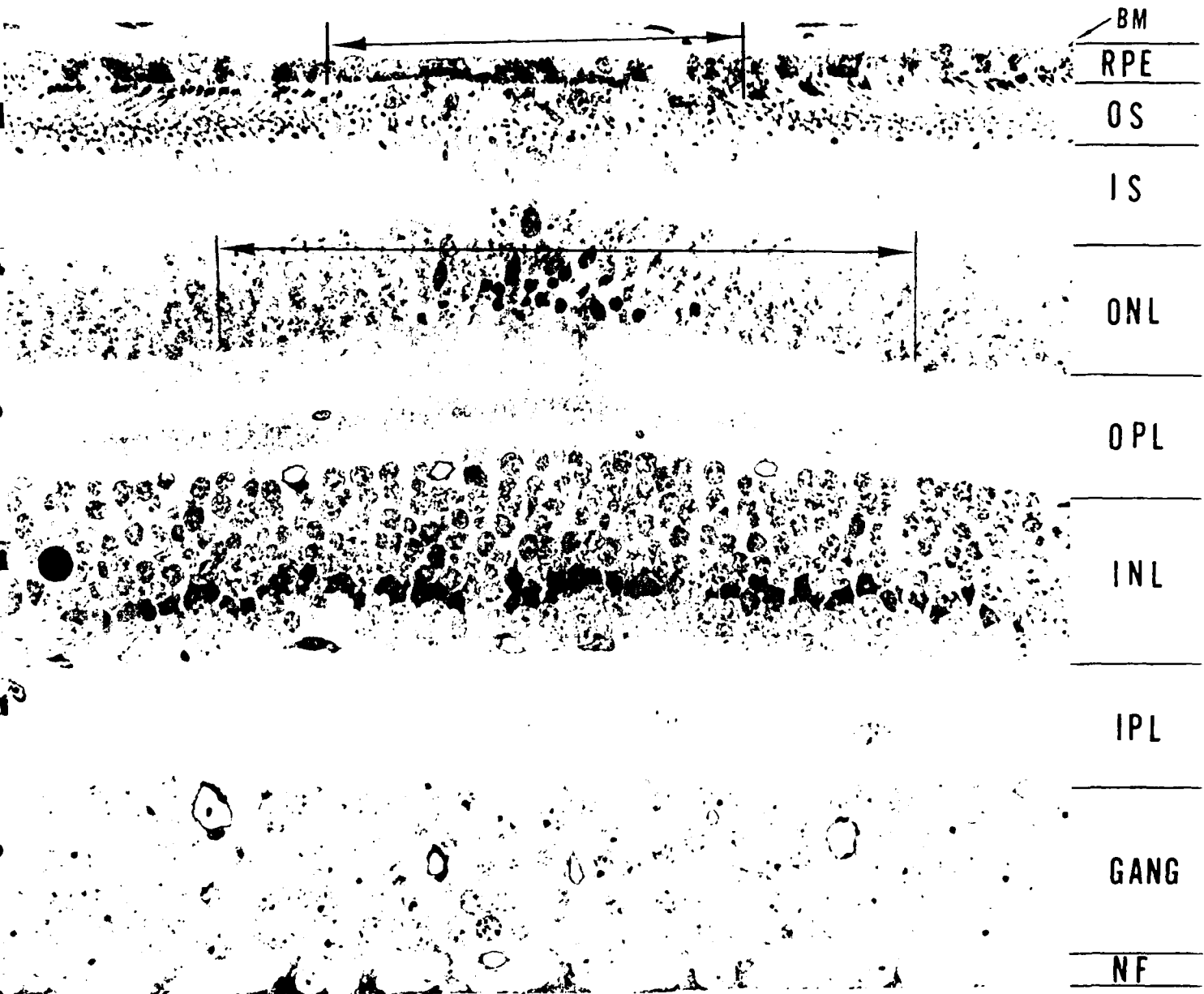
FIGURE 4B





FIGURE 4A

1,000X



BM

RPE

OS

IS

ONL

OPL

INL

IPL

GANG

NF

FIGURE 4

500X

BM	BRUCH'S MEMBRANE
RPE	RETINAL PIGMENT EPITHELIUM
OS	OUTER SEGMENT LAYER
IS	INNER SEGMENT LAYER
ONL	OUTER NUCLEAR LAYER
OPL	OUTER PLEXIFORM LAYER
IPL	INNER PLEXIFORM LAYER
GANG	GANGLION CELL LAYER
NF	NERVE FIBER LAYER

REPRODUCED AT GOVERNMENT EXPENSE

INNER SEGMENTS OF RODS & CONES; OUTER LIMITING MEMBRANE; OUTER NUCLEAR LAYER

There is a curvilinear zone of outward bowing of the outer limiting membrane averaging 207 microns in width (range 180-300 microns) (Fig. 4). Within this zone the inner segments of the rods and cones are slightly foreshortened. They vary in number from 2-15 in a given one micron thick section. The location of the pyknotic nuclei suggests that they are related to rods rather than cones.

OUTER PLEXIFORM LAYER

In several of the marker lesions this layer appears to bow slightly outward. Dark staining material is observed within axons outside the site of the lesion (Fig. 8). This material may be derived from the pyknotic nuclei in the outer nuclear layer.

EXPERIMENTAL LESIONS

The histologic alterations in the left eye are qualitatively similar to those observed in the marked lesions. Each of the left eye experimental lesion are consistent with respect to size and configuration. In effect they resemble a smaller version of the marker lesions. The morphologic alterations in the right eye (in the area in which the lesions were placed) are equivocal and may or may not represent valid tissue responses to coherent radiation exposure.

EXPERIMENTAL LESION - LEFT EYE R.P.E.

The width of the zone of R.P.E. alteration varies from 70 to 90 microns (average 83 microns) (Fig. 9). In all other respects the cellular alterations are identical to those observed in the marker lesions (Fig. 9A).

OUTER SEGMENTS OF RODS AND CONES

Disruption and distortion of the rod and cone outer segments is seen in all lesions. The width of the zone of damage averages 75 microns. In each lesion one micron thick sections can be seen to contain one displaced cell presumed to be derived from the RPE. The displaced cells appear distorted and are morphologically identical to those observed in greater numbers in this location in the marker lesions.

INNER SEGMENTS OF RODS & CONES; OUTER LIMITING MEMBRANE; OUTER NUCLEAR LAYER

The inner segments of the rods and cones within the lesion appear slightly foreshortened and there is a corresponding slight outward bowing of the outer limiting membrane over a zone averaging 100 microns in width. The outer nuclear layer contains pyknotic nuclei (up to 6/one micron thick section) located near the junction of the outer nuclear with the outer plexiform layer.

MORPHOLOGY OF MARKER & EXPERIMENTAL LESIONS

The parameters of the histologic alterations are remarkably consistent in each of the marker lesions (both eye) and in each of the experimental lesions in the left eye. We could detect only equivocal histologic evidence of the experimental lesions in the right eye.

MARKER LESIONS

The histologic alterations are limited to the retinal pigment epithelium, the outer and inner segments of the rods and cones, the outer nuclear layer and the outer plexiform layer. The inner retinal layers do not appear to be involved. Bruch's membrane and the choriocapillaries show no evidence of injury. Occasional cells resembling macrophages are seen in the choriocapillaris underlying the retinal lesions. No other evidence of inflammation is observed.

RETINAL PIGMENT EPITHELIUM

The width of the area of RPE alteration varies from 130-200 microns (average 158 microns). The RPE layer within the lesion is composed of cells that are flatter than those in the surrounding area. There is a relatively sharp border between the lesions and the surrounding RPE evidenced by intralesional loss of pigment granules from the apical aspect of the RPE cells (Fig. 4). The outer nuclear layers contain pyknotic nuclei located primarily in the zone adjacent to the outer plexiform layer (Fig. 4B). The intralesional RPE cells are otherwise unremarkable and average 13 microns in diameter. An occasional cell within a lesion appears binucleated, but this phenomenon is also seen in untreated areas. No mitotic figures are seen. There is a close (apparently normal) relationship between the basal infoldings of the intralesional RPE cells and the pigment epithelial basement membrane (Fig. 5). Widening of this interface is observed only in occasional cells.

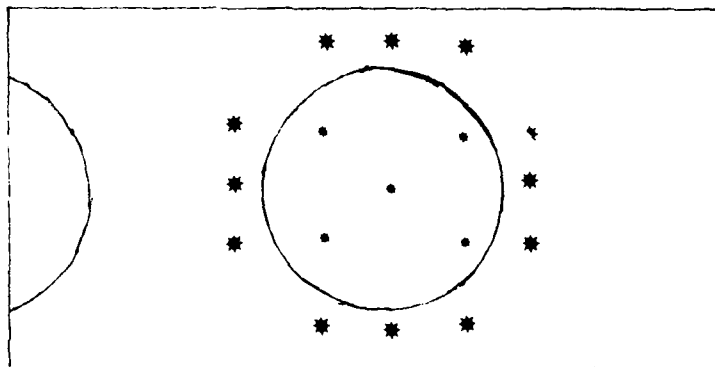
OUTER SEGMENTS OF THE RODS AND CONES

Disruption and distortion of rod and cone outer segments is present in all marker lesions (Fig. 6). The width of the zone of damage is slightly less than that seen in the RPE (average 140 microns). This layer also contains displaced cells that appear to be derived from the RPE. These vary in number from one to six in a given one micron section through a lesion (Fig. 7). The displaced cells are severely distorted and appear to be partially degenerated. Their nuclei are morphologically similar to those seen in the RPE and their cytoplasm contains elongated spindle-shaped pigment granules and membrane-bound material resembling outer segment lamellae. It cannot be determined whether this material has been assimilated in the cytoplasm of these cells before or after the cells were displaced into the outer segment area.

FIGURE 3 T I E OF EXPERIMENTAL EXPOSURES (SINGLE PULSE)

<u>Lesion #</u>	<u>O.S.</u>	<u>O.D.</u>
1	36 uJ	9.9 uJ
2	23	9.0
3	33	8.6
4	29	9.0
5	32	11.0
	<hr/>	<hr/>
AVERAGE	30.6	9.5

LESION PLACEMENT IN MACULAR REGION



* = Marker Lesion

• = Experimental Lesion

FIGURE 2

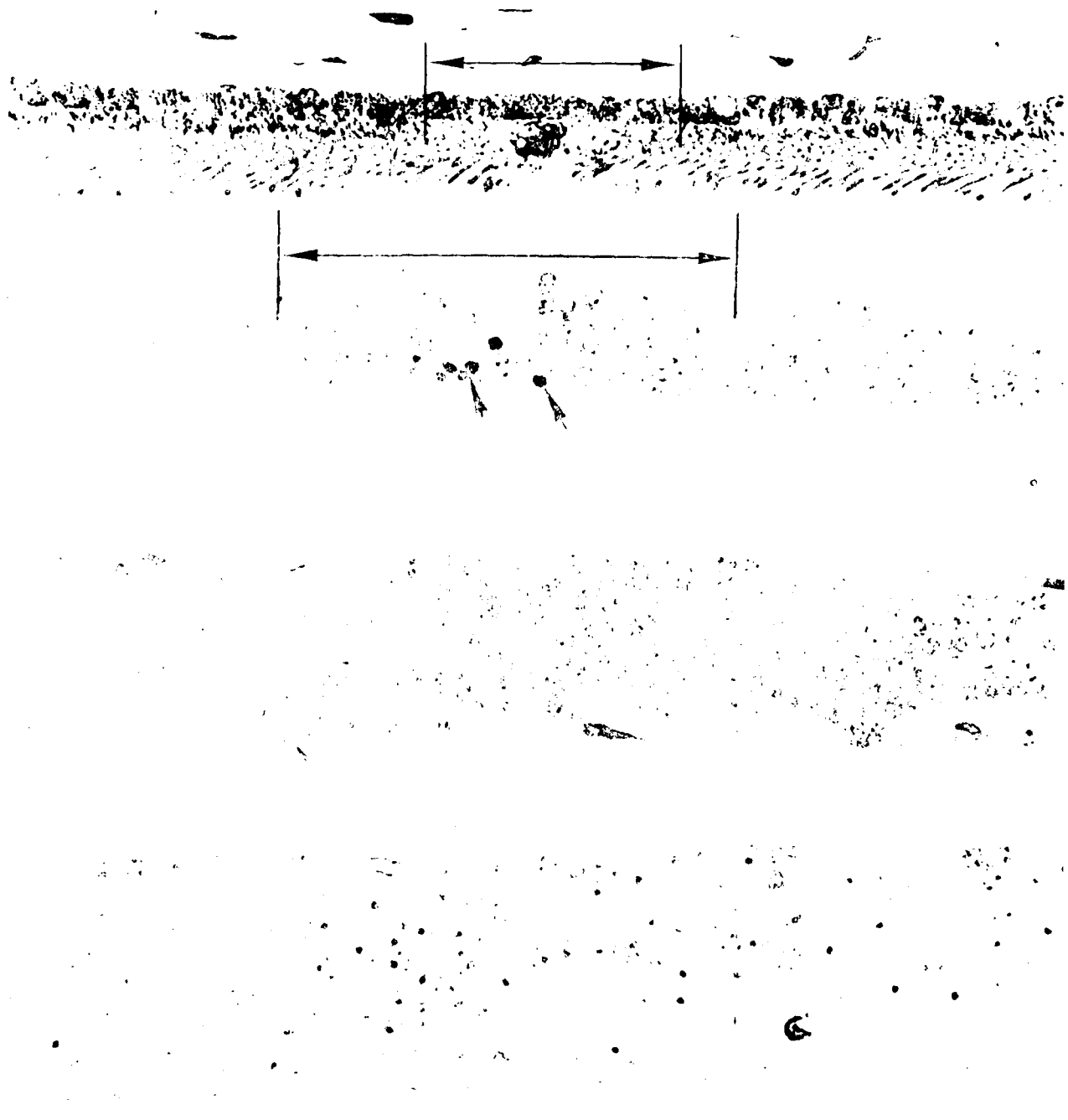


FIGURE 9

500X

FIGURE 9 EXPERIMENTAL LESION LEFT EYE

ALTERATIONS ARE IDENTICAL TO THOSE SEEN IN MARKER LESIONS. BRACKETED AREAS DEPICT ZONES OF RPE OUTER SEGMENT & OUTER NUCLEAR LAYER ABNORMALITIES. ARROWHEADS POINT TO PYKNOTIC NUCLEI IN THE OUTER NUCLEAR LAYER. NOTE SINGLE DISPLACED RPE CELL AND COMPARE WITH MULTIPLE CELLS OF SIMILAR NATURE IN MARKER LESIONS. FIGURE 9A SHOWS AT HIGHER MAGNIFICATION A DISPLACED CELL WITH MORPHOLOGICAL CHARACTERISTICS OF RPE CELLS.



FIGURE 9A

4,500X

OUTER PLEXIFORM LAYER

This layer appears grossly unaffected. Occasional axons contain dark staining material similar to that observed in the marker lesions.

CENTRAL FOVEOLAR LESION - LEFT EYE

Figures 10, 10A, 10B depict the appearance of this lesion in a one micron thick section. Except for anatomic differences inherent in this region of the retina this lesion is morphologically similar to the other experimental lesions in the parafoveal area that have already been discussed. The central foveolar lesion measures approximately 70 microns in width at the RPE level. This encompasses approximately 23% of the width of the foveolar depression (measured in Fig. 10 at the level of the ganglion cell layer as approximately 300 microns in width).

EXPERIMENTAL LESION - RIGHT EYE

Multiple sections through the area of the retina in which the experimental lesions were placed fail to reveal unequivocal histologic evidence of tissue alteration caused by coherent light exposure at 9.5 microjoules/pulse.

Occasional vacuoles containing unidentified cellular elements are seen in the region of the apical portion of the RPE (Fig. 11). These are consistently seen in each of the areas in which the lesions are placed, but no other recognizable tissue alterations are noted.

DISCUSSION OF RESULTS

The morphologic alterations in each retinal lesion 7 days after exposures to coherent light reflect changes caused by the initial insult combined with those related to tissue repair. Relatively little fixation and processing artifact is present. Based upon the present data the following conclusions seem reasonable:

1.

The marker lesions in each eye, and the experimental lesion in the left eye (30.6 microjoules/pulse) are qualitatively similar but differ in magnitude. At the RPE level, and at the level of the rod and cone outer segments, the width of each experimental lesion is slightly greater than the diameter of the incident single light pulse; while the marker lesions, produced by 120 pulses, vary in width up to 4 times the diameter of the incident light pulses. Thus the diameter of the tissue damage is in part a function of the TIE. At the lower TIE it is at least as wide as the diameter of the incident pulse.

2.

The alterations are limited to the RPE and to the outer retina. The same tissue layers are involved in the marker and in the experimental lesions despite the much greater TIE of the marker lesions.

3.

The flatter appearing RPE cells within the confines of the lesions are believed to be replacement cells that have migrated by amitotic sliding into the injury site from the surrounding RPE. This migration is presumed to have occurred quite soon after the injury. The approximation of the basal infoldings of these cells to their basement membrane, and of the latter to Bruch's membrane, is remarkably similar to that seen in cells surrounding the lesion and in other unexposed portions of the retina. This suggests that "normal" contact between these cells and Bruch's membrane has developed within 7 days. A longitudinal study commencing immediately after laser exposure could provide documentation of the initial damage and of the dynamics of the interval tissue healing responses.

4.

The cells displaced into the rod and cone outer segments appear to be derived from the RPE and to represent the residua of the injured RPE cells. The number of displaced cells is smaller in the experimental lesions than in the marker lesions where more RPE cells seem to have been injured. It is uncertain whether these displaced cells have retained their viability. Their cytoplasmic contents include outer segments lamellar material that could have been assimilated either prior to the injury, or after the cell was displaced.

5.

The damaged outer segments of the rods and cones are still evident 7 days after the injury. It is not possible to state how many outer segments were initially injured nor whether some have regenerated. The density of rod and cone outer segments and their respective diameter is not uniform and varies in different portions of the retina. Thus the degree of change will also vary with the location of the injury.

6.

The pyknotic nuclei in the outer nuclear layer are primarily those of rods. The pyknosis is interpreted as an indirect effect of outer segment degeneration rather than a direct effect of laser injury. A longitudinal study could clarify this observation.

7.

The dark staining material within axons of the outer plexiform layer appears to have been transported from the outer nuclear layer, possibly from the nuclei undergoing pyknosis. This may represent a form of agonal axoplasmic transport occurring in degenerating cells.

8.

The foveolar lesion in the left retina is centered with respect to the foveolar depression. If the morphologic alterations in this lesion signify concurrent loss of function then this lesion could be considered to cause a scotoma involving approximately 23% of the foveolar region.

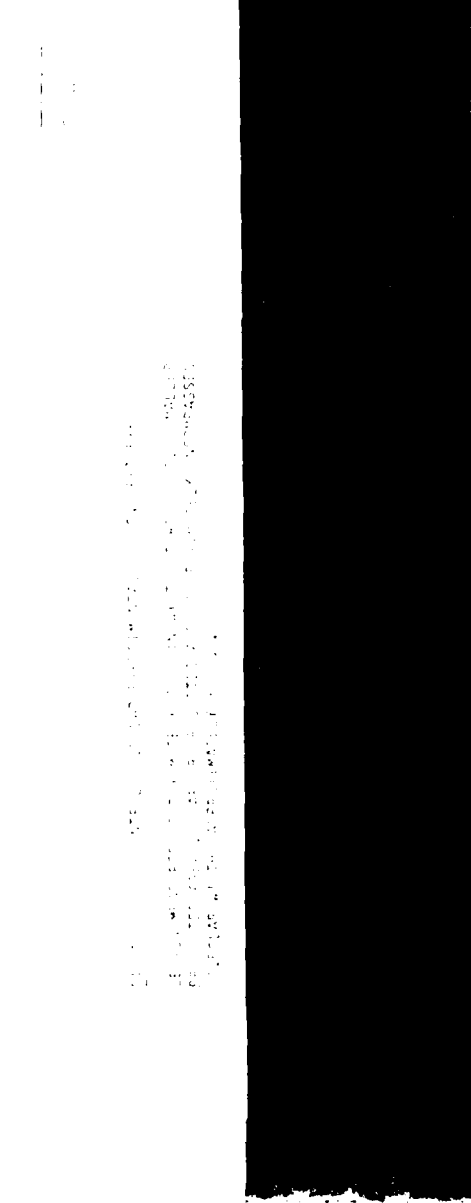
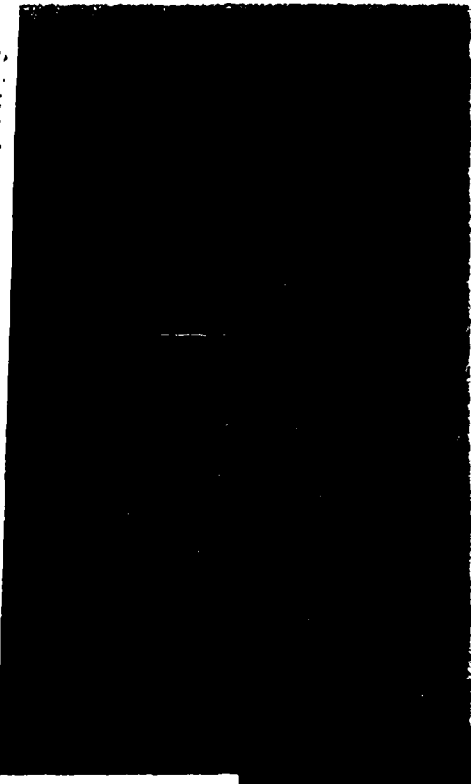
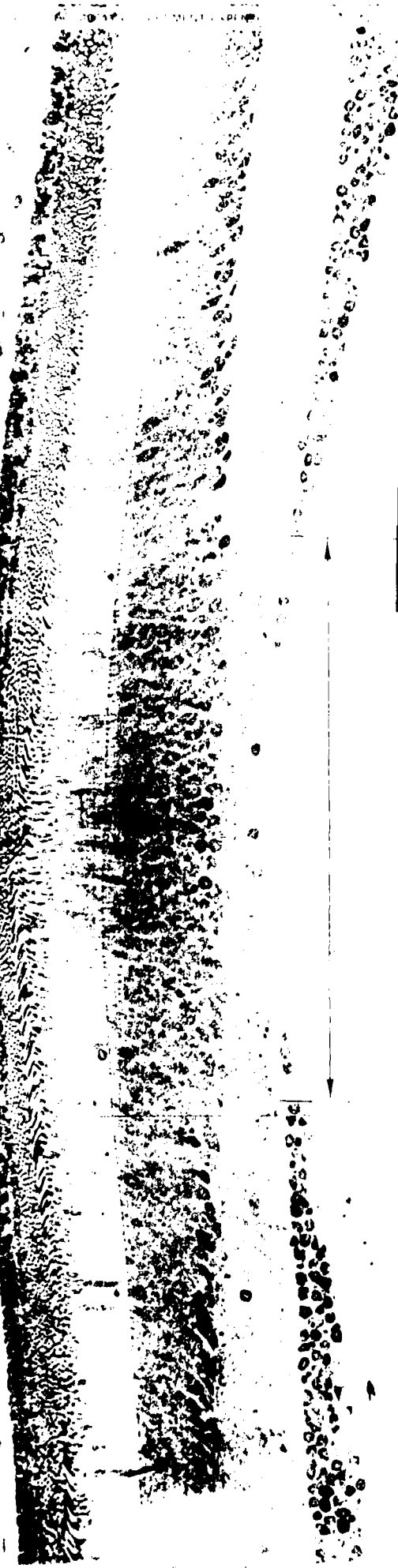
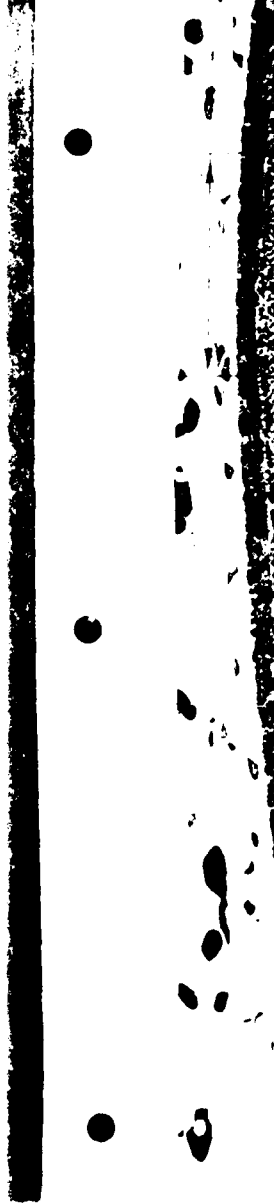
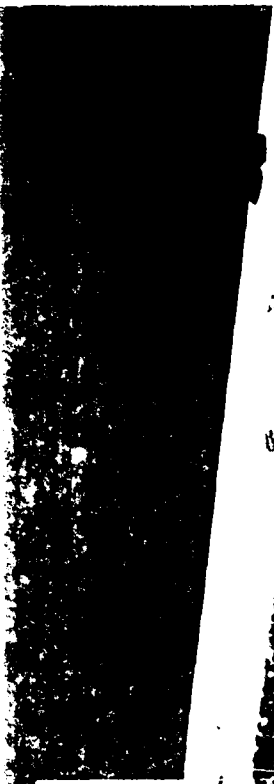




FIGURE 10A HIGHER MAGNIFICATION OF REGION DEPICTED IN FIGURE 10.
ARROWHEAD POINTS TO SINGLE DISPLACED RPE CELL.

FIGURE 10A
1,000X

FIGURE 10B

4,500X

NUC

FIGURE 10B THIN SECTION FROM SAME LESION SEEN IN FIGURES 10 AND 10A.

NOTE BINUCLEATED RPE CELL. DEBRIS LIES WITHIN CYTOPLASM OF DISPLACED RPE CELL LOCATED WITHIN OUTER SEGMENT LAYER.

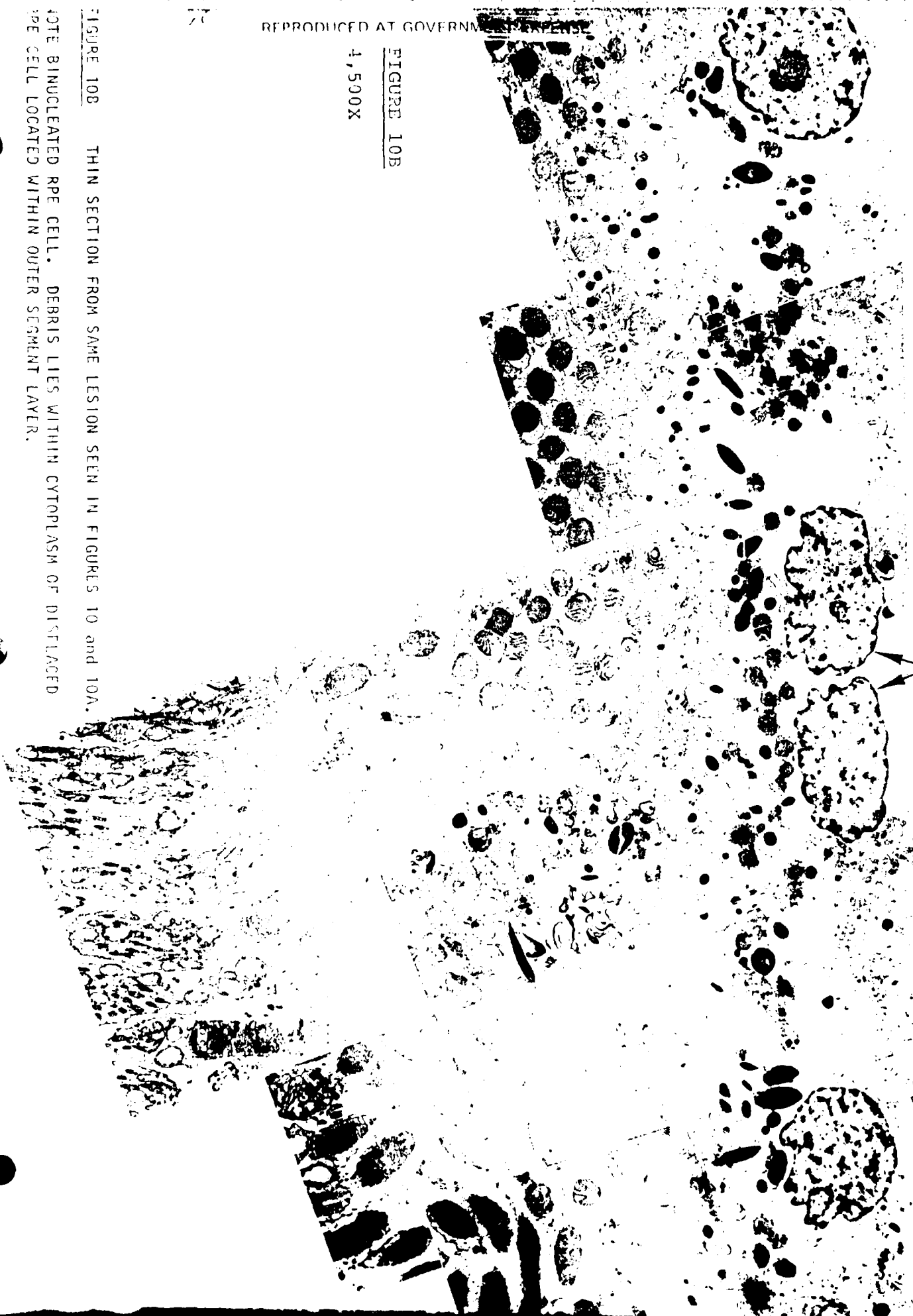


FIGURE 11 EXPERIMENTAL LESION RIGHT EYE

EXCEPT FOR AN OCCASIONAL VACUOLE (ARROW) NO MORPHOLOGICAL ALTERATIONS
ARE OBSERVED IN THE AREA IN WHICH THE LESION WAS PLACED.

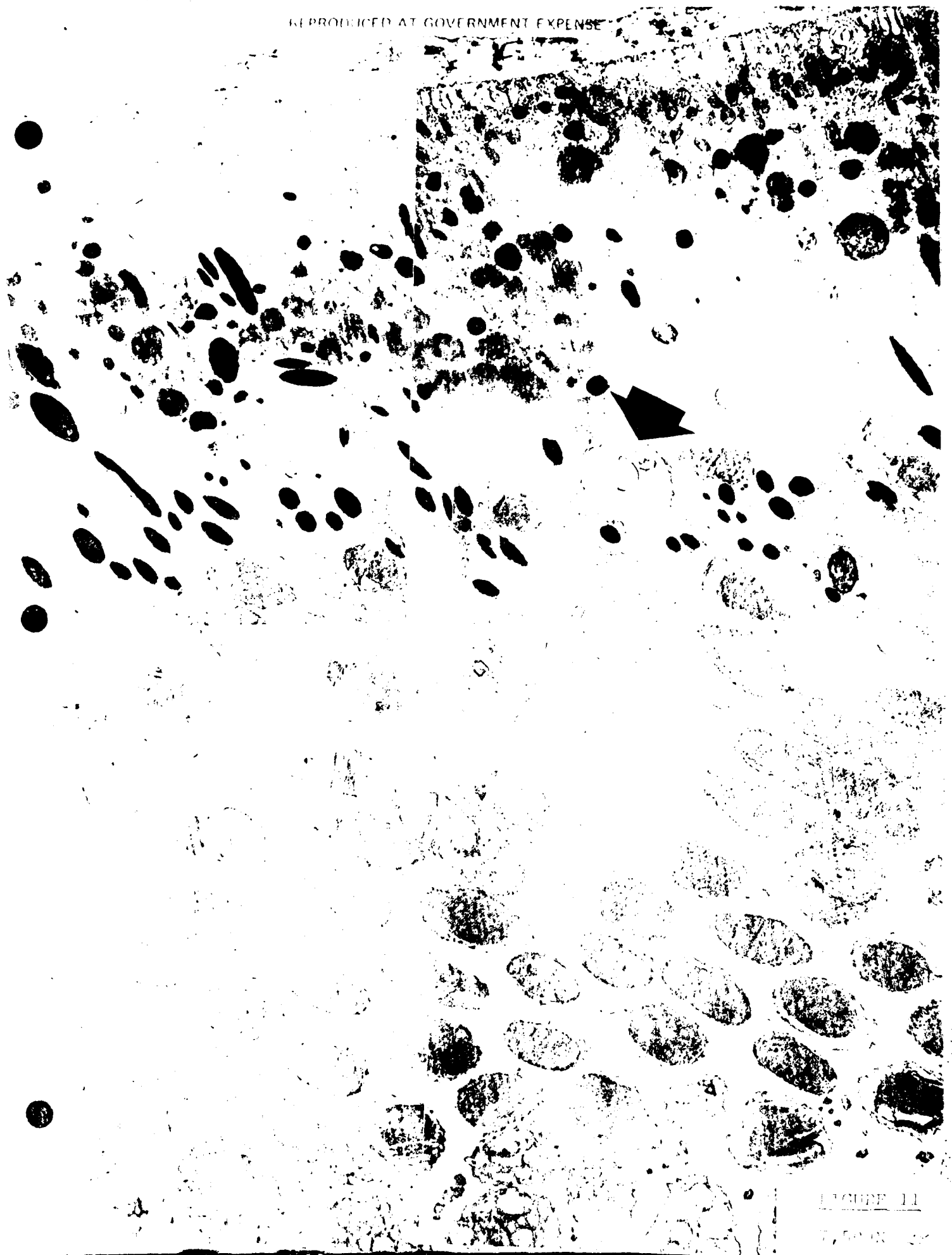


FIGURE 11

7-50X

A 70 micron lesion subtends an angle of 15 minutes of arc at the nodal point of a rhesus eye (assuming the eye to be 20 mm in diameter and the nodal point to lie 16 mm in front of the retina). At a 40 cm reading distance this would produce a scotoma approximately 2 mm in diameter which is roughly the size of a small case typewriter letter. At six meters the scotoma would cover one letter of the 20/60 line of a Snellen eye chart.

9.

The experimental exposures placed in the right eye by single 9.5 microjoule pulses apparently did not produce detectable tissue alterations. The vacuolar changes described above (Fig. 11) are not accompanied by changes in adjacent layers and we suspect that the vacuoles are incidental alterations unrelated to laser exposure. It is also possible that those exposures did produce tissue alterations that have completely healed or that we missed in our serial sections. If indeed lesions were not produced, one may then assume that a single 20 nanosecond 9.5 microjoule pulse or 50 micron spot diameter and wavelength of 900 nm is below the ED50 for this retina, while a 30.6 microjoule pulse is above the ED50.

RECOMMENDATIONS

1.

This study demonstrates that a single 20 nanosecond, 50 micron diameter 30.6 microjoule pulse of coherent light at the gallium arsenide wavelength (900 nm) produces a significant and consistent tissue damage (LD 100). The study also provides equivocal evidence suggesting that a single 9.5 microjoule/pulse of similar dimension and duration will not cause a lesion. Additional studies are recommended which would be directed toward a determination of the minimal TIE (LD 50) necessary to produce histologic evidence of retinal injury. Presumably these would be between 9.5 and 30.6 microjoules/pulse.

2.

The present investigation delineates the tissue alterations present 7 days after laser exposure. A longitudinal study is recommended which will be designed to characterize the sequential interval and late histologic changes occurring in the retina after laser exposure at a TIE of 30.6 microjoules/pulse or higher. An attempt should be made to identify the parameters of the lesion immediately after exposure, and at intervals thereafter (e.g., 4 hours, 12 hours, 2, 4, 14 and 28 days). This would permit a better assessment of the dimensions of the immediate visual perturbation that might occur in an individual inadvertently exposed to laser energy, and would also provide data regarding the reparative tissue responses, the potential for recovery of visual function and its timing.

DISTRIBUTION LIST

4 copies

Commander
 Letterman Army Institute of
 Research (LAIR), Bldg. 1110
 ATTN: SGRD-ULZ-RC
 Presidio of San Francisco, CA 94129-6815

4 copies

Commander
 US Army Medical Research and Development Command
 ATTN: SGRD-RMS
 Fort Detrick, Frederick, Maryland 21701-5012

12 copies

Defense Technical Information Center (DTIC)
 ATTN: DTIC-DDAC
 Cameron Station
 Alexandria, VA 22304-6145

1 copy

Dean
 School of Medicine
 Uniformed Services University of the
 Health Sciences
 4301 Jones Bridge Road
 Bethesda, MD 20814-4799

1 copy

Commandant
 Academy of Health Sciences, US Army
 ATTN: AHS-CDM
 Fort Sam Houston, TX 78234-6100

END

FILMED

5-85

DTIC